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Weissbach, H., and

rk, Stony Brook; the

Research Section, Department of Medicine, Albert Einstein Medical Center, Philadelphia; the Medical Department, Brookhaven National Laboratory, Upton, NY, and the Roche Institute of Molecular Biology, Nutley, NJ.

PROTEASES AND LUNG INJURY: A STATE-OF-THE-ART MINIREVIEW

This minireview of ongoing knowledge about the relationship between proteases and lung injury is presented in the following sections: Historical Perspectives; Highlights of Recent Developments (1. Mononuclear Phagocytes; Role in Elastase Homeostasis, 2. Oxidative Inactivation of Elastase-inhibitors, and 3. Immunochemical Assays of Elastin Degradation); Current Major Questions; and Some Suggestions for Future Study. In summary, it is widely supposed today that the destructive changes in lung parenchyma associated with pulmonary emphysema are mediated in large part by unrestrained proteolytic activity in lung connective tissue. This condition is thought to arise whenever elastolytic proteases are released from lung cells and are ineffectively downregulated by endogenous proteinase inhibitors in the lower respiratory tract. Marginated neutrophils, resident alveolar macrophages, and (more recently) freshly recruited mononuclear leukocytes in transit between blood capillaries and alveolar airspaces have been implicated as likely sources of lung-damaging elastases. On the other hand, alpha$_1$-proteinase inhibitor (α_1Pi) has been implicated as an important regulator of neutrophil and monocyte elastases. The hypothesis that an imbalance between these factors, favoring protease activity, is pathogenetic in emphysema rests largely on the signal observations of Laurell and Eriksson who recognized that selected cases of early-onset familial emphysema were associated with heritable α_1-Pi deficiency, and Gross *et al.* who showed that intrapulmonary instillation of elastolytic proteases produced anatomic derangements in experimental animals characteristic of human emphysema. Recent progress in this area has been exciting and has been the subject of discussion at several major symposia over the last decade. However, protease-antiprotease imbalance as the mechanism of alveolar effacement in most forms of emphysema still remains to be proven.

Janoff, A.

CHEST 83(5):54S-58S, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York, Stony Brook.

CIGARETTE SMOKE BLOCKS CROSS-LINKING OF ELASTIN *IN VITRO*

For the assay system reported here, purified tropoelastin and lysyl oxidase were incubated for 24 hrs. to facilitate tropoelastin coacervation and the resultant desmosine was measured by a previously described radioimmunoassay method. Three different kinds of cigarette smoke solution were tested in this assay system. Results of the five experiments described here showed that no desmosine was detected under baseline conditions. However, using complete enzyme-substrate mixtures and incubating at 37° C in the absence of smoke, desmosine formation was demonstrated both with homologous enzyme-substrate mixtures and heterologous mixtures. On the other hand, in the presence of aqueous smoke solutions, desmosine synthesis was inhibited in all five experiments. When an independent assay method was used, an inhibitory effect of gas

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

phase cigarette smoke on the chemical reaction which precedes and is required for desmosine formation was also shown. In these experiments, lysyl oxidase catalyzed oxidative deamination of lysine ϵ-amino groups was monitored. The effect of gas phase smoke on elastin oxidation was dose-dependent, and inhibition was readily detectable even at dilutions of smoke solution as high as 1:150. Oxidation of lysine ϵ-amino groups precedes synthesis of all known elastin cross-links. Thus, the data presented here support the conclusion that cigarette smoke can inhibit formation of other elastin crosslinks besides desmosine.

Laurent, P., *Janoff, A.* and Kagan, H. M.

CHEST 83(5):63S-65S, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York, Stony Brook, and the Department of Biochemistry, Boston University School of Medicine, Boston.

LEVELS OF ELASTASE ACTIVITY IN BRONCHOALVEOLAR LAVAGE FLUIDS OF HEALTHY SMOKERS AND NONSMOKERS

The purpose of this study was to compare elastase levels in lung washes of healthy volunteer smokers and nonsmokers and to partly characterize any enzymes found with respect to their chemical classification and cellular source(s) within the lung. In order to do this, elastase activity was measured in concentrated, cell-free bronchoalveolar lavage (BAL), using the synthetic substrate butyloxycarbonyl-L-alanyl-L-alanyl-L-prolyl-L-valyl-amino-methylcoumarin. The BAL fluids obtained from young, asymptomatic smokers with normal urine desmosine concentrations one hour after they had smoked two cigarettes showed significant increases in elastase levels compared with those in nonsmoking control subjects. Repeated BAL samples were obtained at later times from one smoker with a high initial enzyme value and from one nonsmoking control subject. Elastase activity varied over time, but both subjects consistently remained within their respective group ranges. Inhibition studies on pooled BAL from smokers showed that the elastase activity present had properties of both serine and metalloenzymes, suggesting that neutrophils and/or monocytes (serine enzyme) as well as macrophages (metalloenzyme) contributed to the observed activity. Lung lavage cells obtained from two of the smokers and two of the nonsmokers were stained with both a chromogenic substrate and by indirect immunofluorescence for the serine enzyme. The results of these and other studies show some asymptomatic smokers have significantly more elastase activity in their bronchopulmonary secretions than do nonsmokers (as measured with a low molecular weight synthetic substrate). Also, the enzyme activity recovered in smokers' BAL appears to be derived mainly from neutrophils (serine enzyme) and macrophages (metalloenzyme), rather than from monocytes.

Janoff, A., Raju, L. and Dearing, R.

American Review of Respiratory Disease 127(5):540-544, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York at Stony Brook, and the Pulmonary Disease Section, Nassau County Medical Center, East Meadow, NY.

THE ROLE OF OXIDATIVI

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Janoff, A. et al.

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From the Department of Patho

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Janoff, A.

Journal of Applied Physiology:
1983.

Other support: National Heart.

From the Department of Patho
Stony Brook.

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

THE ROLE OF OXIDATIVE PROCESSES IN EMPHYSEMA

Both proteases and activated species of oxygen have been implicated as mediators of immunologic lung injury, and the present report attempts to link these two pathways of connective tissue destruction. For instance, elastase/elastase inhibitor imbalance in the lung has been implicated in the pathogenesis of pulmonary emphysema. In light of this, it may be significant that the activity of two major elastase inhibitors, alpha 1-proteinase inhibitor (alpha 1-antitrypsin, α_1PI) and bronchial mucus proteinase inhibitor, can be decreased by oxidizing agents. The effect can be observed with ozone, substances present in cigarette smoke, and oxygen metabolites generated by lung macrophages as well as peroxidative systems released by other phagocytic cells. Thus, α_1PI recovered from lung washings of cigarette smokers has only half the predicted normal activity per mg inhibitor and contains four moles of methionine sulfoxide (oxidized methionine) per mole of inactive inhibitor. By contrast, α_1PI purified from nonsmokers' lung washings is fully active and contains only native methionine. At the same time, lung washes from some smokers show significantly greater hydrolytic activity against a specific synthetic elastase substrate than do lung washes of nonsmokers. In addition to the possible effect of cigarette smoking on lung elastase/elastase inhibitor balance, it seems smoking also may interfere with elastin repair mechanisms. Specifically, it has been seen that acidic water-soluble gas phase components of cigarette smoke prevent synthesis of desmosine cross-links during elastinogenesis *in vitro*.

Janoff, A. et al.

American Review of Respiratory Disease 127(2):S31-S38, 1983.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York, Stony Brook.

BIOCHEMICAL LINKS BETWEEN CIGARETTE SMOKING AND PULMONARY EMPHYSEMA

It is widely believed today that the destructive changes associated with emphysema are mediated in large part by unrestrained proteolytic (especially elastolytic) activity in lung connective tissue. It is further argued that this last condition results from the heightened release of elastolytic proteases by cells in the lung, coupled with an acquired or genetic deficiency in the endogenous antiproteases responsible for regulating these enzymes. Even though this hypothesis still requires rigorous proof, newer information is rapidly developing that links the protease-pathogenesis model to an environmental risk factor associated with disease, namely cigarette smoking. A triad of chemical and cellular effects produced by cigarette smoke has been suggested as contributing to altered elastin metabolism and eventual development of emphysema in smokers. The present article reviews some of these observations, seeks to place them within the overall framework of the protease model and attempts to raise questions for future study.

Janoff, A.

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 55(2):285-293, 1983.

Other support: National Heart, Lung and Blood Institute.

From the Department of Pathology, State University of New York at Stony Brook, Stony Brook.

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

PROTEASES AND ANTIPROTEASES IN THE LUNG

Proteases and antiproteases in the lung have received increasing attention in the past 15 years because of growing evidence that an imbalance between proteolytic enzymes and their inhibitors may give rise to certain features of chronic obstructive lung diseases (COLD) in man. For example, unrestrained elastolytic activity in the lung has been suggested to be responsible for the alveolar deformation seen in pulmonary emphysema, while several workers have shown that elastolytic attack also causes alterations in the conducting airways similar to those associated with chronic bronchitis. The first section of this paper is devoted to proteases in the lung (proteases of the alveolar macrophage and of the polymorphonuclear neutrophil (PMN)) and to potential targets of macrophage and PMN elastases in lung connective tissue. Following sections consider antiproteases in the lung, elevation of lung proteases in smokers, depression of lung antiproteases in smokers, inactivation of antiproteases by oxidants in tobacco smoke, and inactivation of antiproteases by cell-generated oxidants. Summation of this detailed review chapter shows that some of the elastolytic proteases in the lung and some of the major lung antiproteases that regulate the activity of these enzymes are discussed. The investigators then propose potential mechanisms by which cigarette smoking could elevate the elastase "burden" of the lung and/or depress the lung's elastase-inhibitor "screen." Finally, the protease-pathogenesis model of COLD is reexamined in an attempt to show how cigarette smoking and other environmental and genetic factors might influence the balance between proteases and antiproteases in the lung.

Janoff, A. and Carp, H.

In: Newball, H. H. (ed.): *Immunopharmacology of the Lung*, New York:Marcel Dekker, Inc., 1983, pp. 173-208.

Other support: National Heart, Lung and Blood Institute.

From the Department of Pathology, State University of New York, Stony Brook.

CROSS-CIRCULATION STUDIES ON THE INFLUENCE OF HYPOXIA AND HYPOXEMIA OF NEUROEPITHELIAL BODIES IN YOUNG RABBITS

Using young rabbits for testing, the reactions of neuroepithelial bodies (NEB) to (1) hypoxia with normoxemia in the arteria pulmonalis on the one hand, and (2) hypoxemia in the arteria pulmonalis with normoxic aeration on the other hand, were investigated by means of cross-circulation experiments and light microscopic, electron microscopic and morphometric techniques. Results showed that hypoxically aerated young rabbits, which received normoxemic blood in their arteria pulmonalis from a donor rabbit by means of an arterio-arterial cross-circulation with mutual exchange transfusion, revealed an increased exocytosis of the dense-core vesicles of their NEB. Normoxically aerated young rabbits which received hypoxemic blood in an identical manner, did not exhibit an increased exocytosis. In light of these results, it is concluded that the NEB apparently react directly to the hypoxia of the inhaled air and not to the hypoxemia of the pulmonary blood. By the release of serotonin and a polypeptide substance, they may produce a local vasoconstriction in hypoxically aerated lung areas, enabling an intrapulmonary regulation of the V/Q ratio. This is regarded as additional proof that the NEB, while being modulated by the CNS, probably are

intrapulmonary chemoreceptors
sition of the inhaled air.

Lauweryns, J. M. et al.

Cell and Tissue Research 193:3

Other support: Nationaal Fond

From the Laboratory of Histopa
Medicine, Leuven, Belgium.

INTERACTION OF SEROTOI
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Sheller, J. R., Holtzman, M. J.

Journal of Applied Physiology:
1982.

Other support: National Institut

From the Cardiovascular Researc
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REFLEX STIMULATION OF T
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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

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intrapulmonary chemoreceptors with local secretory activities, reacting to the composition of the inhaled air.

Lauweryns, J. M. et al.

Cell and Tissue Research 193:373-386, 1978.

Other support: Nationaal Fonds voor Wetenschappelijk Onderzoek, Belgium.

From the Laboratory of Histopathology, Katholieke Universiteit te Leuven School of Medicine, Leuven, Belgium.

INTERACTION OF SEROTONIN WITH VAGAL- AND ACh-INDUCED BRONCHOCONSTRICTION IN CANINE LUNGS

In this attempt to determine whether serotonin acts at the level of the airway smooth muscle muscarinic receptor, a comparison was made of the effect of serotonin on bronchoconstriction caused by vagal stimulation and by inhalation of acetylcholine (ACh) aerosols. To do this, the bronchoconstrictor response to electrical stimulation of the peripheral ends of both cut cervical vagus nerves was potentiated by serotonin aerosols in 10 experiments in seven anesthetized dogs. The bronchoconstrictor response to acetylcholine aerosols was unchanged after serotonin. Specifically, the bronchoconstriction caused by electrical stimulation of vagal nerves was significantly increased by pretreatment with serotonin, but the response to inhaled ACh was unchanged. The potentiation of the effect of vagal stimulation did not result from alteration in base-line airway caliber, because base-line R_L was not changed by serotonin aerosols. Because of these results, it was concluded that the smooth muscle muscarinic receptor is not the site where serotonin interacts with the vagal motor pathway to airway smooth muscle. It seems, rather, that serotonin acts at the level of the parasympathetic ganglia or the postganglionic nerve terminal to potentiate the bronchoconstrictor response to vagal stimulation.

Sheller, J. R., Holtzman, M. J., Skoogh, B-E., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 52(4):964-966, 1982.

Other support: National Institutes of Health.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

REFLEX STIMULATION OF TRACHEAL MUCUS GLAND SECRETION BY GASTRIC IRRITATION IN CATS

This study had two aims: (1) to determine whether flow from the submucosal glands of the trachea is reflexly regulated by sensory stimuli from the stomach in the cat, and (2) if such a gastropulmonary reflex exists, what sensory and motor pathways are important. Responses tested here included mechanical stimulation, blockade of the secretory response and atropine sulfate infusion followed by electrical stimulation of the central end of either vagus nerve. Results showed that mechanical stimulation of the gastric mucosa increased submucosal gland secretions from 7.9±0.7 to 17.4±1.7

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

nl/min (mean±SE, $P<0.001$). This effect was prevented reversibly by cooling both abdominal vagus nerves to −3° C before stimulation and was restored by rewarming the nerves. The effect was prevented irreversibly by cutting both abdominal vagus nerves and was then mimicked by electrically stimulating the central cut end of one of the nerves. This increase in secretions caused by electrical stimulation of the nerve was prevented by administration of atropine sulfate before stimulation. It was concluded, therefore, that stimuli from the stomach reflexly affect the rate of submucosal gland secretion. The sensory limb of this reflex lies in the abdominal vagus nerves, and the motor pathways are mediated by cholinergic muscarinic receptors.

German, V. F., Corrales, R., Ueki, I. F., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 52(5):1153-1155, 1982.

Other support: National Heart, Lung, and Blood Institute and Vick Divisions Research and Development.

From the Cardiovascular Research Institute and Departments of Medicine, Pediatrics and Physiology, University of California, San Francisco.

SELECTIVE EFFECT OF GENERAL ANESTHETICS ON REFLEX BRONCHOCONSTRICTOR RESPONSES IN DOGS

Although anesthesia may be critical in the study of bronchoconstrictor responses and, in particular, the study of reflex responses, the sites of action of general anesthetics in the reflex pathway are unclear. Therefore, in this attempt to determine which part of the parasympathetic bronchoconstrictor pathway is most sensitive to depression by general anesthetics, the authors stimulated different parts of the pathway in dogs after initial anesthesia with chloralose and urethan and then after additional anesthetic drugs. The entire reflex pathway was stimulated by producing apnea or hypoventilation, the sensory pathway by electrically stimulating the proximal ends of cut superior laryngeal nerves, and the motor pathway by stimulating the distal end of a cut cervical vagus nerve. Bronchoconstrictor responses to all stimuli were assessed with a bypassed tracheal segment. When no additional anesthetic was administered, responses to all stimuli increased with time. Small additional doses of anesthetics (thiopental, 1-5 mg/kg; pentobarbital, 1-2 mg/kg; amobarbital, 1-2 mg/kg; or chloralose, 10 mg/kg) decreased responses to reflex and sensory stimulation markedly and reversibly, but they did not affect responses to motor stimulation. Increased doses decreased responses to motor stimulation as well. While a previous study showed that barbiturates depress parasympathetic ganglionic synapses, this study suggests that central nervous system synapses may be even more sensitive to depression by general anesthetics.

Holtzman, M. J., Hahn, H. L., Sasaki, K., Skoogh, B.-E., Graf, P. D., Fabbri, L. M., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 53(1):126-133, 1982.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

DOSE-DEPENDENT INHIBI
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Sheppard, D., Epstein, J., Holt

Journal of Applied Physiol: Resp

Other support: National Heart.
sources Board.

From the Cardiovascular Researc
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General Hospital, San Francisc

BARBITURATES DEPRESS V
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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

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Graf, P. D., Fabbri, L.

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DOSE-DEPENDENT INHIBITION OF COLD AIR-INDUCED BRONCHOCONSTRICTION BY ATROPINE

Inhalation of cold air is a well-known cause of bronchoconstriction in people with asthma. Observations like this have recently triggered a number of investigations dealing with bronchoconstriction induced by cold air or by exercise and inhibited by treatment with atropine or antimuscarinic agents. The study presented here was undertaken to determine whether inhalation of atropine could inhibit cold air-induced bronchoconstriction in a dose-dependent fashion. In seven subjects with asthma the authors assessed the effects of placebo and of various doses of inhaled atropine (0.13-2.08 mg) on base-line specific airway resistance (sRaw) and on the increase in sRaw produced by 5 min of voluntary eucapnic hyperventilation with subfreezing air at −17° C. They also assessed the effect of the lowest dose of atropine on the increase in sRaw produced by five breaths of 1.0% methacholine. Atropine in doses of 0.13 or 0.26 mg caused a maximal reduction in base-line sRaw and completely inhibited the effect of 1.0% methacholine on sRaw, but it did not inhibit the bronchomotor response to cold air. Higher doses of atropine did inhibit the effect of cold air on sRaw in a dose-dependent fashion. The dose of atropine required to inhibit this effect of cold air varied with the increase in sRaw produced by cold air after placebo. These results suggest that cold air causes bronchoconstriction through vagal pathways and that higher doses of antimuscarinic agents may be required to inhibit vagally mediated bronchoconstriction than those required to reduce base-line airway tone or to inhibit the effects of a large dose of an inhaled muscarinic agonist.

Sheppard, D., Epstein, J., Holtzman, M. J., *Nadel, J. A.*, and Boushey, H. A.

Journal of Applied Physiol: Respirat. Environ. Exercise Physiol. 53(1):169-174, 1982.

Other support: National Heart, Lung, and Blood Institute and California Air Resources Board.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco; and Medical Service, San Francisco General Hospital, San Francisco.

BARBITURATES DEPRESS VAGAL MOTOR PATHWAY TO FERRET TRACHEA AT GANGLIA

Barbiturates have been shown before to depress the bronchomotor response to stimulation of the vagus nerve. In the present attempt to determine which site in the vagal motor pathway to airway smooth muscle is most sensitive to depression by barbiturates, isometric muscle tension *in vitro* was recorded and the vagal motor pathway at four different sites before and after exposure to barbiturates was stimulated. In isolated tracheal rings from ferrets, muscarinic receptors in the neuromuscular junction were stimulated by exogenous acetylcholine, postganglionic nerve fibers by electrical field stimulation, and the postsynaptic membrane in ganglia by 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP). A tracheal nerve-muscle preparation to stimulate preganglionic fibers in the vagus nerve electrically was also developed. Activation of ganglia by DMPP or by vagus nerve stimulation was depressed by barbiturates at 10-fold lower concentrations than those depressing the activation of postganglionic nerves or the neuromuscular junction. These findings suggest that the postsynaptic membrane in parasympathetic ganglia is the site in the vagal motor pathway most sensitive to depression by barbiturates.

1002318929

Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

Skoogh, B.-E., Holtzman, M. J., Sheller, J. R., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 53(1):253-257, 1982.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

INTRAVENOUS VERSUS INHALED ATROPINE FOR INHIBITING BRONCHOCONSTRICTOR RESPONSES IN DOGS

In the comparative study presented here, the effect of the muscarinic antagonist atropine sulfate given by two routes, intravenous and inhaled, was assessed. Bronchoconstrictor responses were monitored in anesthetized dogs by determining the increase in total pulmonary resistance before and after increasing doses of atropine and then constructing inhibition dose-response curves. Results showed that there can be a marked difference between the inhibitory effects of atropine on bronchoconstrictor responses to inhaled acetylcholine and to acetylcholine released by vagal stimulation and that the difference depends only on the route of atropine administration. When atropine was administered as an aerosol for inhalation, there was more potent inhibition of the bronchoconstrictor responses to inhaled acetylcholine than of the bronchoconstrictor responses to vagal stimulation. By contrast, when atropine was administered intravenously, there was equivalent inhibition of both stimuli. Therefore, in studies designed to inhibit bronchoconstriction due to an inhaled muscarinic agonist to the same degree as bronchoconstriction due to vagal reflex, atropine might better be given intravenously than by inhalation.

Holtzman, M. J., McNamara, M. P., Sheppard, D., Fabbri, L. M., Hahn, H. L., Graf, P. D., and *Nadel, J. A.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 54(1):134-139, 1983.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

REGULATORY PEPTIDES IN THE MAMMALIAN RESPIRATORY TRACT

The full respiratory tracts of 11 adult cats, eight Wistar rats, and six Duncan Hartley guinea pigs were isolated and subjected to radioimmunoassay (RIA) and immunocytochemistry in an attempt to map and quantify the presence of five peptides, bombesin, cholecystokinin (CCK), SRIF, substance P, and vasoactive intestinal polypeptide (VIP), in various areas of the respiratory tracts. VIP and substance P were found in higher concentrations than CCK, SRIF and bombesin. In general, higher concentrations were found in the main airways than in lung tissue. Immunocytochemistry localized VIP and substance P to autonomic nerves, whereas bombesin was found in very scattered mucosal endocrine cells of the cat bronchial epithelium. CCK

and SRIF could not be convi
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both health and disease.

Ghatei, M. A., Sheppard, M.
G. P., *Polak, J. M.*, and Bloc

Endocrinology 111(4):1248-125

Other support: Medical Resea

From the Departments of Medi
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OXYGEN-RADICAL-MEDIA VASOCONSTRICTION IN IS

The investigation presente
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Tate, R. M., Vanbenthuysen, K
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American Review of Respirator

Other support: Kroc, Hill, Sw
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From the Webb-Waring Lung In
ter, Denver.

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

and SRIF could not be convincingly localized by immunocytochemistry because of their low tissue concentrations. In addition, species differences were noted, with high VIP but low substance P concentrations in the main airways of the cat; this relationship was reversed in the rat and guinea pig. Gel permeation chromatography of extracts revealed one peak, coeluting with the pure standard peptide, for CCK-8, SRIF, substance P, and VIP. Bombesin immunoreactivity was separable into two peaks, the major of which corresponded in position to that of gastrin-releasing peptide and a later minor peak to the position of the bombesin standard. Thus, the finding of five potently active peptides in the respiratory tract of these three species strongly suggests that they have a regulatory role in respiratory function. In fact, many recent studies indicate that the respiratory tract has in it the elements of an elaborate peptidergic control system that may conceivably have a significant role in the control of respiratory function in both health and disease.

Ghatei, M. A., Sheppard, M. N., O'Shaughnessy, D. J., Adrian, T. E., McGregor, G. P., *Polak, J. M.*, and Bloom, S. R.

Endocrinology 111(4):1248-1254, 1982.

Other support: Medical Research Council, UK, and the Wellcome Foundation.

From the Departments of Medicine and Histochemistry, Hammersmith Hospital, London.

OXYGEN-RADICAL-MEDIATED PERMEABILITY EDEMA AND VASOCONSTRICTION IN ISOLATED PERFUSED RABBIT LUNGS

The investigation presented here demonstrates that chemically-generated O_2 intermediates can cause increased alveolar-capillary membrane (ACM) permeability and vasoconstriction in isolated lungs. In order to identify this specific interaction of O_2 radicals and lung tissue, it was necessary to test the effects of O_2 radicals in the absence of other toxic substances such as neutrophil or platelet products. The O_2 radicals generated by xanthine oxidase caused protein-rich edema and increases in lung perfusion pressures that were inhibitable by catalase (hydrogen peroxide scavenger) or dimethylthiourea (hydroxyl radical scavenger) but not by superoxide dismutase. To determine the effect of O_2 radicals on ACM permeability without interference from increased perfusion pressures, papaverine was used to maintain baseline perfusion pressures during O_2 radical exposure and then ACM integrity was assessed by evaluating the response of isolated lungs to elevated outflow pressures (10mm Hg for 10 min). Under these conditions, increased ACM permeability manifested by weight gains and lavage albumin accumulations occurred in lungs treated with xanthine oxidase but not in control lungs. It was concluded, therefore, that O_2 radicals can cause increased ACM permeability and vasoconstriction in isolated lungs.

Tate, R. M., Vanbenthuysen, K. M., Shasby, D. M., McMurtry, I. F., and *Repine, J. E.*

American Review of Respiratory Disease 126:802-806, 1982.

Other support: Kroc, Hill, Swan and Kleberg Foundations, National Institutes of Health, American Heart Association and American Lung Association of Colorado.

From the Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

10023193

Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

ACETYL GLYCERYL ETHER PHOSPHORYLCHOLINE-STIMULATED HUMAN PLATELETS CAUSE PULMONARY HYPERTENSION AND EDEMA IN ISOLATED RABBIT LUNGS

The occurrence of macrophages, neutrophages, pulmonary platelet microthrombi, and intrapulmonary platelet sequestration in the adult respiratory distress syndrome (ARDS) suggests that platelets may contribute to this form of acute edematous lung injury. Because stimulated human macrophages and neutrophils can release acetyl glyceryl ether phosphorylcholine (AGEPC), a potent platelet activator, it was hypothesized that in ARDS, leukocyte release of AGEPC might stimulate platelets to release thromboxane A_2 (TXA_2), which then produces pulmonary hypertension and lung edema. In support of this premise, the authors found that pulmonary hypertension and edema occurred in isolated rabbit lungs perfused with human platelets and AGEPC, but not with platelets or AGEPC alone. Infusion of a vasodilator (nitroglycerin) to maintain base-line pulmonary artery pressures in lungs perfused with platelets and AGEPC prevented the development of lung edema, suggesting that platelet- and AGEPC-induced edema was hydrostatic in nature. Additional experiments suggested that the increased pressure was a result of TXA_2 release from platelets stimulated by AGEPC. Specifically, preincubation of platelets with imidazole, a thromboxane synthetase blocker, prior to infusion with AGEPC significantly diminished pulmonary hypertension and prevented lung edema. The role of TXA_2 was further suggested when perfusates from lungs infused with platelets and AGEPC developed high levels of TXA_2, whereas perfusates from controls did not. These results suggest that platelet aggregation induced by AGEPC may contribute to ARDS by releasing TXA_2, which raises microvascular pressure and increases edema formation, especially when an underlying permeability defect is present.

Heffner, J. E., Shoemaker, S. A., Canham, E. M., Patel, M., McMurtry, I. E., Morris, H. G., and *Repine, J. E.*

Journal of Clinical Investigation 71:351-357, 1983.

Other support: American Heart Association of Colorado, National Institutes of Health, American Lung Association of Colorado, and Kroc, Hill, Swan, and Kleberg Foundations.

From the Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

KINETIC ANALYSIS OF RESPIRATORY TRACT PROTEINS RECOVERED DURING A SEQUENTIAL LAVAGE PROTOCOL

This paper presents an attempt to analyze the dynamic recovery of specific protein substances from the bronchoalveolar space during a sequential lavage protocol. Although bronchoalveolar lavage (BAL) has been used as a research tool for over a decade, the technique of lavage has varied markedly between laboratories. For example, lavage instillate volumes from 50 to 300 ml have been used, and yet the influence of the variable of total lavage volume on subsequent protein recovery is uncertain. In this study, a sequential lavage protocol was performed on 14 normal individuals and the recovered aliquots were analyzed individually for the absolute and relative concentrations of several protein substances. These proteins included free secretory component and secretory IgA, which emanate from airway secretions, and IgG, which is thought

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Merrill, W., O'Hearn, E., F

American Review of Respir

Other support: Veterans Ac

From the West Haven Veter
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Ryan, U. S. and Ryan, J. W.

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Other support: U. S. Public H

From Department of Medicine

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to transude from more distal alveolar sites. Analysis of these data showed a marked decrease in the absolute concentration of all proteins measured in serial aliquots. Analysis of protein ratios in sequential aliquots, however, revealed no significant change from the first to the fifth recovered aliquot. Finally, the influence of the size of the first recovered aliquot on absolute and relative concentrations of proteins was measured. Here there seemed to be a trend indicating preferential recovery of airway proteins in smaller aliquots. This was significant for the ratio of free secretory component to albumin. It was concluded that lung proteins are efficiently and homogenously sampled with 100 ml of lavage instillate. Larger volumes will add more protein but not alter protein ratios. Lavage with smaller volumes may preferentially sample airway proteins.

Merrill, W., O'Hearn, E., Rankin, J., Naegel, G., Matthay, R., and *Reynolds, H. Y.*

American Review of Respiratory Disease 126:617-620, 1982.

Other support: Veterans Administration.

From the West Haven Veterans Administration Medical Center, West Haven, CT, and the Department of Medicine, Yale University, New Haven, CT.

ANGIOTENSIN-CONVERTING ENZYME: II. PULMONARY ENDOTHELIAL CELLS IN CULTURE

One of the technical limitations to progress in understanding of specific functions of a particular cell type is the availability of that cell type in pure culture. Although over the past 12 years a number of different specific metabolic activities have been attributed to pulmonary endothelial cells, by and large these activities were based on conclusions drawn from indirect evidence. In this reported attempt to be able to examine more directly for specific metabolic activities, a program was started to obtain pulmonary endothelial cells in culture. Two methods were developed: (1) cells can be obtained from pulmonary artery and vein of large animals (cow, pig), and (2) cells can be obtained from the microvasculature of small animals (rat, guinea pig, and rabbit). The latter technique can also be used to obtain cells from a lobe of lung from large animals and may be adaptable for use with human tissue. In the first technique, pulmonary arteries, free of blood, are filled with collagenase in Puck's saline for 25 min. The collagenase mixture containing cells is removed and centrifuged; the pellet is then resuspended and seeded into culture flasks. In the second method, lungs are perfused (artery to vein) with Krebs-Henseleit solution until the effluent is blood-free. Collagenase is introduced, and the lungs are then perfused in the opposite direction (vein to artery) until the flow stops spontaneously (ca. 15 min). The detached cells are collected and seeded as before. The cells possess converting enzyme activity and are reactive with antibodies to converting enzyme, Factor VIII and α_2-macroglobulin. The cells synthesize prostaglandins and related substances. In addition, they possess ADPase and synthesize angiotensin-converting enzyme.

Ryan, U. S. and Ryan, J. W.

Environmental Health Perspectives 35:171-180, 1980.

Other support: U. S. Public Health Service and the John A. Hartford Foundation, Inc.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

USE OF MICROCARRIERS TO ISOLATE AND CULTURE PULMONARY MICROVASCULAR ENDOTHELIUM

Among the challenges for endothelial cell culture are (1) development of means of harvesting cells from different tissues and from different sized vessels of a single tissue, and (2) development of means of cell harvest and passage that preserve and do not damage irremediably cell surface structures. The latter has been accomplished for endothelium of large vessels but not for small vessels until now. In the present study, however, means have been devised for collecting and culturing endothelial cells of pulmonary arterioles of approximately 40-60 μm in diameter. The procedure is illustrated by collection of endothelial cells from rabbit pulmonary pre-capillary vessels. The lungs are perfused free of blood with physiological saline and then cold (4°C) saline (containing EDTA and microcarriers 600/ml; 40-60 μm diameter) is perfused via the pulmonary artery. The direction of flow is reversed periodically to collect the bead-cell harvest from the arterial side. Cold shock and EDTA cause the endothelial cells to detach from the vessels under conditions such that the cells remain attached to the microcarriers. The cells are collected on the microcarriers still in the presence of a chelating agent but in the absence of proteolytic enzymes. It is believed that this approach is sufficiently general to allow the collection of endothelium from arterioles and venules of similar size in any tissue. Further, by using microcarriers of different sizes, it should be possible to extend the approach so that one can obtain cultures of endothelia representative of vessels of all sizes.

Ryan, U. S. et al.

Tissue & Cell 14(3):597-606, 1982.

Other support: National Institutes of Health.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

PROLIFERATION OF PULMONARY ENDOTHELIAL CELLS: TIME-LAPSE CINEMATOGRAPHY OF GROWTH TO CONFLUENCE AND RESTITUTION OF MONOLAYER AFTER WOUNDING

In a regular culture, seeded at low density, all of the cells divide and continue to do so until confluence is reached. Artificial "wounding" (denudation) of a confluent monolayer of pulmonary artery endothelial cells elicits a regenerative response, resulting in the reformation of a continuous single layer of endothelium. Maintenance of this monolayer is important both for the exchange of nutrients and for interactions between blood solutes and endothelial enzymes and transport systems. In the study presented here, time-lapse cinematography was used to compare proliferative behavior of bovine pulmonary endothelial cells in (1) establishment of a monolayer from a low-density seed and (2) restitution of a confluent monolayer following a mechanical wound (removal of cells from an area 5 × 15 mm by scraping). Culture 2 was not refed after wounding. In culture 2, approx. 30% of the cells accounted for repopulation (confluence in 40 hr). In culture 1, all cells entered into division. Participating cells of culture 2 began division immediately (69 divisions/filmed area in 10 hr. *vs.* four divisions in culture 1). Interdivision times (IDT) were longer and relatively constant in culture 1 until near confluence; none were < 10 h, whereas in 2, 24% of the IDT's were ≤ 10 hr. Remarkably, IDTs of culture 2 decreased steadily until confluence was re-

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Ryan, U. S. et al.

Tissue & Cell 14(4):637-

Other support: National

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Ryan, U. S., Whitney, P.

Journal of Appl. Physiol.

Other support: National

From Department of Med

EFFECTS OF BRADYK
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As a prelude to studi
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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

established. Cell migration in culture 1 was multidirectional while direction of migration in culture 2 was always into the wound area. Mean migration rate (MIG) in culture 2 was related to the site of origin of the cells. Neither culture formed more than a single layer of cells. Although the cell kinetics of cultures 1 and 2 differed, the same goal, confluence, was achieved in either case.

Ryan, U. S. et al.

Tissue & Cell 14(4):637-649, 1982.

Other support: National Institutes of Health.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

LOCALIZATION OF CARBONIC ANHYDRASE ON PULMONARY ARTERY ENDOTHELIAL CELLS IN CULTURE

On the basis of past histological studies, it appears that pulmonary endothelial cells, but not epithelial cells, possess carbonic anhydrase having access to plasma solutes. This work shows specifically that bovine pulmonary artery endothelial cells in culture possess carbonic anhydrase activity and immunoreactivity. Specific immunofluorescence is obtained here when the cells are incubated with rabbit antibovine erythrocyte carbonic anhydrase B and then with goat antirabbit immunoglobulin G coupled to fluorescein. At the level of electron microscopy, antibodies to carbonic anhydrase B are reactive with sites along the plasma membrane and associated caveolae. Multivesicular bodies are the only intracellular sites labeled and appear to correspond to the globular sites of intracellular immunofluorescence. Cells maintained and propagated in culture in the absence of an exogenous source of carbonic anhydrase nonetheless possess carbonic anhydrase activity, suggesting that the cells are capable of synthesizing the enzyme. Taken together, these results indicate that pulmonary artery endothelial cells possess carbonic anhydrase situated so that the enzyme could readily catalyze the dehydration of plasma HCO_3^- to facilitate CO_2 excretion and participate in the regulation of blood pH as central venous blood is converted into systemic arterial blood.

Ryan, U. S., Whitney, P. L., and Ryan, J. W.

Journal of Appl. Physiol.: Respirat. Environ. Exercise Physiol. 53(4):914-919, 1982.

Other support: National Heart, Lung and Blood Institute.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

EFFECTS OF BRADYKININ ON PULMONARY ENDOTHELIAL CELLS IN CULTURE

As a prelude to studies to examine for specific receptors for bradykinin (BK) on pulmonary endothelial cells, these investigators began to examine for the effects of BK on the cyclic nucleotide contents of pulmonary endothelial cells pretreated with a

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

phosphodiesterase inhibitor. To do this, endothelial cells were harvested from bovine mainstem pulmonary artery and were maintained and passaged in culture. Cells were then prepared for assays, which were performed using kits obtained from New England Nuclear Corp. Angiotensin converting enzyme (ACE) was assayed using [^{3}H]benzoyl-Phe-Ala-Pro as substrate. Results showed an apparent sensitivity of bovine pulmonary artery endothelial cells to BK in concentrations at or near those believed to occur physiologically. Equally impressive was the corresponding finding that BK, but none of its lower homologs, exerted effects on the release of arachidonate or its metabolites. Des-Arg^1-BK, at 10^{-7}M, exerted weak effects. Des-Arg^9-BK was not effective even at 10^{-6}M. Similarly, the products formed by ACE, namely the 1-7 and 1-5 homologs of BK, were completely inactive. Hence, there was remarkable specificity in terms of BK and its metabolites. These and other results make it clear that pulmonary endothelial cells are responsive to BK in reasonable concentration. Thus, it is likely that efforts to examine these cells in culture for high affinity binding sites, characteristic of receptors as opposed to metabolic enzymes, will yield affirmative results.

Ryan, U. S., Lehotay, D. C. and Ryan, J. W.

Advances in Experimental Medicine and Biology 156B:767-774, 1983.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL, and the Department of Medicine, University of Pittsburgh, Pittsburgh.

A RADIOASSAY FOR CARBOXYPEPTIDASE N

A simple radioassay is described for carboxypeptidase N (CPN), an enzyme that occurs in plasma and on vascular endothelial cells and epithelial brush border. Since CPN has been reported to be a kininase enzyme that participates in the metabolism of one or more of the anaphylatoxins, this report not only describes the assay but looks at some of the ways that the assay can be used further to explore whether CPN is a physiologically important kininase enzyme or merely, in this context, an enzyme capable of degrading bradykinin *in vitro* but not *in vivo*. In one part of this study, CPN was tested for its reactivity with [^{3}H]benzoyl-Phe-Arg. In another part, a series of experiments were performed in which 2-mercaptomethyl-3-guanidinoethylthiopropionic acid (MGP) was added to the reaction mixture as an inhibitor of CPN. Results showed that [^{3}H]benzoyl-Phe-Arg might be a useful substrate for carboxypeptidase N (and presumably carboxypeptidase B). The substrate can be used conveniently in either *in vitro* or *in vivo* assays. When used as described here for *in vivo* assays, hydrolysis is owing primarily to the tissue-bound enzyme (*e.g.*, that on endothelial cells) and not to the soluble enzyme of plasma.

Ryan, J. W., Chung, A. and *Ryan, U. S.*

Advances in Experimental Medicine and Biology 156B:867-874, 1983.

Other support: U. S. Public Health Service.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

STIMULATED HUMAN I
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The New England Journal

Other support: Elsa U. Par
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From the Hematology-Onco
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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

STIMULATED HUMAN PHAGOCYTES PRODUCE CYTOGENETIC CHANGES IN CULTURED MAMMALIAN CELLS

It has been known that the occurrence of sister-chromatid exchanges in response to diverse physical and chemical agents correlates strongly with the induction of mammalian mutagenicity or carcinogenicity or both. In the study reported here, it is shown that stimulated phagocytes can cause cytogenetic changes in mammalian cells by means of an oxygen-mediated process. Using the sister-chromatid-exchange assay, it was seen that there was a concentration-dependent increase in sister-chromatid exchanges in cultured Chinese hamster ovary cells incubated with leukocytes activated by phorbol myristate acetate. A particulate activator of oxygen metabolism—opsonized zymosan—was also effective, although not to the same extent. The difference is ascribable to the fact that at the concentrations used in this study, opsonized zymosan is a less potent stimulating agent for leukocytic oxidative metabolism than phorbol myristate acetate. These results prove that leukocyte-generated oxygen metabolites or their reaction products with target-cell components can reach the nucleus of the cultured mammalian cell and cause cytogenetic damage—an observation that may help explain the relation between chronic inflammatory states and cancer *in vivo*.

Weitberg, A. B., Weitzman, S. A., Destrempes, M., Latt, S. A., and *Stossel, T. P.*

The New England Journal of Medicine 308(1):26-30, 1983.

Other support: Elsa U. Pardee Foundation, the Ladies' Auxilary to the Veterans of Foreign Wars, Edwin W. Hiam, the American Cancer Society, and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Clinical Genetics Division, Children's Hospital Medical Center, Harvard Medical School, Boston.

CA^{2+} CONTROL OF ACTIN FILAMENT LENGTH: EFFECTS OF MACROPHAGE GELSOLIN ON ACTIN POLYMERIZATION

Gelsolin, a calcium-binding protein of rabbit lung macrophages, confers calcium sensitivity to the regulation of the network structure of actin filaments. In the presence of micromolar calcium concentrations, gelsolin shortens actin filaments (F-actin) without decreasing appreciably the total mass of filaments, leading to a decrease in the gel structure of actin filaments. Since gelsolin is bound to the shortened filaments, it is likely that gelsolin resides at one or both ends of the filaments to prevent them from annealing. The paper presented here identifies by electron microscopy the end of the actin filament to which gelsolin binds, and shows that gelsolin promotes the nucleation of actin to produce a population of short filaments similar to that obtained when gelsolin is added directly to F-actin. In these respects, gelsolin closely resembles a number of other agents such as the cytochalasins, β-actinin, villin, fragmin, and the *Acanthomoeba castellani* capping protein that have been shown to enhance the nucleation of actin and in some cases to shorten actin filaments, to bind one end of the filaments and to reduce the actin network structure. The identification of such a class of functionally analagous actin-modifying agents suggests that the control of actin filament length is of considerable importance for the regulation of cell structure and movement. As is pointed out here, the calcium-dependent shortening of actin filaments is the primary mechanism for the dissolution of an actin gel by gelsolin. Therefore, the ability of gelsolin to produce short filaments irrespective of the initial state of assembly

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

of the actin offers flexibility for controlling the network structure of the cytoplasm in which either the monomeric or polymeric form of actin molecules might predominate at different times.

Yin, H. L., Hartwig, J. H., Maruyama, K., and *Stossel, T. P.*

The Journal of Biological Chemistry 256(18):9693-9697, 1981.

Other support: U. S. Public Health Service.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

SOME PERSPECTIVES ON THE VISCOSITY OF ACTIN FILAMENTS

The existence of actin filaments at the periphery of many cells has encouraged extensive study of the solution properties of F-actin. In the paper presented here, measurements of the dynamic viscosity of various actin filament preparations under conditions of low and controlled shear: (a) confirm the shear rate dependence of F-actin viscosities and show that this dependence obeys the power law relationship observed for entangled synthetic polymers; (b) permit estimation of the extent to which shear artifact amplifies changes in the apparent viscosity of F-actin measured in a falling ball viscometer; (c) show that gel-filtration chromatography of actin and the addition of cytochalasin B to F-actin bring about small (20-40%) changes in the viscosity of the F-actin solutions. These variations are consistent with alterations in the actin-binding protein concentrations required for incipient gelation, a parameter inversely related to average filament length. Therefore: (a) changes in the viscosity of F-actin can be magnified by use of the falling ball viscometer, and may exaggerate their biological importance; (b) chromatography of actin may not be required to obtain meaningful information about the rheology of actin filaments; (c) changes in actin filament length can satisfactorily explain alterations in F-actin viscosity exerted by cytochalasin B and by chromatography, obviating the need to postulate specific interfilament interactions.

Zaner, K. S. and *Stossel, T. P.*

The Journal of Cell Biology 93:987-991, 1982.

Other support: U. S. Public Health Service and the Whitaker Health Science Fund.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

ACTIN GELATIN AND THE STRUCTURE OF CORTICAL CYTOPLASM

This paper summarizes the evidence, based to a large extent on these investigators' studies of proteins isolated from mammalian phagocytic leukocytes, that the nature of molecules that cross-link actin and the control of the length distribution of actin filaments are profoundly important for the regulation of the consistency of actin filaments *in vitro* and probably *in vivo*. Also summarized here is evidence that the gel structure proposed to exist in the cortex of many cells is pertinent to several physiologic phenomena; one specificially emphasized is cytoplasmic movement. In a section entitled, CONSISTENCY OF ACTIN IN CYTOPLASM, the following parameters are discussed: Rheology of Actin Filaments, Gelation of Actin Filaments, Regulation of the Actin Sol-Gel Transformation by the Control of Filament Length, and Does Capping

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Stossel, T. P. et al.

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of Actin Filaments *Per Se* Affect Actin Consistency Independently of Length Changes of the Capped Filaments? The last two sections of this paper are devoted to ACTIN CONSISTENCY AND CYTOPLASMIC STRUCTURE AND FUNCTION and STRUCTURE OF CORTICAL CYTOPLASM. In summary, this paper proposes that cortical cytoplasm of phagocytic leukocytes and probably many other mammalian cells is a branching network of actin filaments cross-linked by actin-binding protein. The consistency of this network is regulated by calcium-sensitive and calcium-insensitive proteins that act on the actin-filament length distribution. Consistency changes in the cortical actin have many consequences for cell function, one of which is the control of cytoplasmic movement.

Stossel, T. P. et al.

Cold Spring Harbor Symposia on Quantitative Biology 46:569-578, 1982.

Other support: U. S. Public Health Service, the Edwin S. Webster Foundation and Edwin W. Hiam.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

CALCIUM CONTROL OF ACTIN NETWORK STRUCTURE BY GELSOLIN

This book chapter reviews a system which depends on calcium for the control of actin structure. The general principle behind this system is that calcium regulates the *average length* of actin filaments and can thereby control their viscosity and rigidity. The components of this system were first identified in rabbit lung macrophages which can be considered a prototype of highly motile cells. Therefore, this principle of the regulation of cell motility is likely to be applicable in general to other motile eukaryotic cells. In considering the structure of the cortical cytoplasm, it is noted here that the motor of the macrophage appears to reside in the peripheral cytoplasm beneath the plasma membrane. Under light microscopy, the cortical region excludes organelles contributing to the gel-like appearance of the cytoplasm. The thickness of this cortical region changes during various cell activities. Calcium regulation of actin filament length and consequently cytoplasmic rigidity is discussed in a section on regulation of actin gel-sol transformation. Another section is devoted to calcium regulation of actin filament by gelsolin, a 91,000-dalton, heat-labile, globular protein, which shortens actin filaments when activated by calcium in the micromolar concentration range contributing to the collapse of their three-dimensional lattice. When the ambient calcium concentration is decreased, the regulator is inactivated and the actin gel reforms. Overall, information currently available permits a biochemical explanation of calcium-regulated directional movements of the cytoplasm. Calcium activates a regulatory protein, gelsolin, shortening actin filaments. This causes a localized breakdown in actin structure, creating a gradient of cytoplasmic rigidity, and directional movement follows from the resultant imbalance in tension generated by myosin.

Yin, H. L. and *Stossel, T. P.*

In: Cheung, Y. W. (ed.): *Calcium and Cell Function*, New York: Academic Press, 1982, Vol. 2, pp. 325-337.

Other support: U. S. Public Health Service, Edwin S. Webster Foundation, Edwin W. Hiam, and Charles L. and Jane D. Kaufman.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

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FLOW-VOLUME CURVES IN INFANTS WITH LUNG DISEASE

This study was undertaken to investigate the feasibility of using a new partial expiratory flow-volume (PEFV) technique in infants with a variety of lung diseases and to determine whether such measurements provide additional information on the severity of lung disease, site of obstruction and response to treatment. As reported here, PEFV maneuvers have been performed on nine occasions on six infants with a variety of pulmonary problems using the new technique for thoracic compression. The infants were placed in an inflatable bag that was itself within a canvas bag. By sudden controlled inflation of the inner bag at end inspiration, PEFV curves were generated and recorded by means of a face mask and pneumotachograph. By comparing these flow results with airway resistance and lung volume measurements obtained from the infants in whole body plethysmography and by noting the effect of inhaling a helium/oxygen gas mixture, it was possible to partition the airway obstruction between large and small airways. The presence of small airway obstruction was noted in the absence of changes in airway resistance or lung volume in several instances. A complete evaluation of airway function should include this test of forced expiration for greater understanding and treatment of lung disease in infancy.

Godfrey, S., Bar-Yishay, E., Arad, I., Landau, L. I., and *Taussig, L. M.*

Pediatrics 72(4):517-522, 1983.

Other support: Fogarty International Scholarship and Glaxo Group Research.

From the Department of Pediatrics, Hadassah University Hospital, Jerusalem.

SYNTHETIC ELASTASE INHIBITORS: PROSPECTS FOR USE IN THE TREATMENT OF EMPHYSEMA

Human leukocyte (HL) elastase is considered to be the enzyme primarily responsible for the destruction of lung tissue observed in pulmonary emphysema. In the review paper presented here, several approaches to treatment of emphysema are discussed. These include (1) use of α_1-Protease Inhibitor (α_1-PI), (2) use of other natural high molecular weight protease inhibitors from either plant or animal sources, (3) use of antioxidants to protect the α_1-PI, and (4) use of low molecular weight elastase inhibitors. Recent research has shown that synthetic elastase inhibitors can be designed to bind either reversibly or irreversibly to elastase. Indeed, a number of potent reversible and irreversible inhibitors have already been developed for human leukocyte elastase. Several of these inhibitors have been shown to be effective at preventing emphysema in animal models of the disease. As of now it is clear that a synthetic elastase inhibitor would be a useful therapeutic agent and that there are excellent prospects for its development for use in treating human emphysema.

Powers, J. C. (*Travis, J.*)

American Review of Respiratory Disease 127:S54-S58, 1983.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

IMMUNOLOGIC MEASUR
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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

IMMUNOLOGIC MEASUREMENT OF ELASTIN-DERIVED PEPTIDES IN HUMAN SERUM

Chronic obstructive pulmonary disease (COPD) develops insidiously over many years, and usually significant impairment of lung function has occurred before the disease is diagnosed. It seems possible that a quantitative test that is capable of identifying the presence of lung damage at an early stage, before symptoms develop, could be useful in identifying those people who are at risk and possibly in monitoring the progression of the disease. It is likely that destruction of the elastin fiber is a prerequisite for the development of COPD, and it is possible that immunologic identification in the serum of peptides derived from lung elastin degradation might be an effective approach to the early detection and monitoring of the disease. These investigators prepared antibodies to peptides derived from human lung parenchymal elastin and used these antibodies in an enzyme-linked immunosorbent assay to quantitate elastin-derived peptides in the serum of 39 normal control nonsmokers, 33 smokers with normal lung function and 40 patients with COPD. On average, statistically significant higher levels of elastin-derived peptides were found in the normal smokers and COPD patients compared to the controls. Further work with large numbers of subjects is necessary to determine whether such a test could be effective in identifying those individuals who are at risk of developing COPD.

Kucich, U., Christner, P., Lippmann, M., Fein, A., Goldberg, A., Kimbel, P., *Weinbaum, G.*, and Rosenbloom, J.

American Review of Respiratory Disease 127:S28-S30, 1983.

Other support: National Institutes of Health.

From the Pulmonary Disease Section, Albert Einstein Medical Center, Philadelphia; the Center for Oral Health Research, Department of Anatomy and Histology, School of Dental Medicine, University of Pennsylvania, Philadelphia; and the Department of Medicine, the Graduate Hospital, Philadelphia.

DYNAMICS OF THE NEUROENDOCRINE CELL—REGULATORY PEPTIDE SYSTEM IN THE LUNG: SPECIFIC OVERVIEW AND NEW RESULTS

This overview of the biology of the lung neuroendocrine (NE) cells includes some review material from earlier studies and certain new observations dealing with oxygen-related changes in NE cell numbers. For the study presented here, changes in argyrophil NE cell numbers, intensity of 5-HT fluorescence, and arterial medial thickness in the lungs of neonate rabbits were assessed under various oxygen treatments. Results of this study showed that NE cell numbers and 5-HT fluorescence in normal rabbits increased from 12 hours before to one day after birth, and NE cells declined thereafter to the 10th day. In acute hypoxic 5-day-old rabbits, 5-HT fluorescence was decreased, whereas NE cell numbers and medial thickness were unchanged. Neonates hypoxic from birth had higher NE cell numbers and increased medial thickness at three and five days, whereas 5-HT fluorescence was decreased compared to normoxic controls. These chronically hypoxic neonates showed a dramatic drop in argyrophil NE cell numbers to below normal when they were exposed to normoxia for one hour, but cell numbers and medial thickness returned to normal at 4 and 24 hours, respectively. The effect of acute and chronic hyperoxia was also tested and results showed that 100% O_2 for 2-2.5 hours caused a significant drop in detectable NE cell numbers, whereas 40% O_2 in N_2 caused no change. Although at the present time the mechanisms and pathways

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Source: https://www.industrydocuments.ucsf.edu/docs/yqhk0000

that tell us how the biogenic amines and polypeptide hormones function in lung control cannot be defined, the accumulated evidence that these substances are present and active implies that their role is vital.

Keith, I. M. and *Will, J. A.*

Experimental Lung Research 3(3&4):387-402, 1982.

Other support: College of Agricultural and Life Sciences, University of Wisconsin-Madison and the Air Force Office of Scientific Research.

From the Department of Veterinary Science, CALS, and the Department of Anesthesiology, Medical School, University of Wisconsin-Madison.

III. *Heart and Circulation*

DISTRIBUTION OF HIGH DENSITY LIPOPROTEIN PARTICLES WITH DIFFERENT APOPROTEIN COMPOSITION: PARTICLES WITH A-I AND A-II AND PARTICLES WITH A-I BUT NO A-II

High density lipoproteins (HDL) have been shown to contain numerous chemically and immunochemically distinct polypeptides referred to as apoproteins (apo). In the study reported here, HDL were subfractionated by equilibrium CsCl gradient centrifugation of the d 1.063-1.21 g/ml HDL fraction isolated from two men and two women. The various HDL subfractions were analyzed for their apo A-I, A-II, B, D, and E and the major lipid contents. ApoA-I and A-II were found throughout the density gradient. ApoE was found in all HDL fractions with the higher concentration in the lower density fractions. Conversely, the concentration of apoD increased as the density of the HDL fraction increased. Each density subfraction underwent quantitative precipitation with anti-A-I and anti-A-II immunoglobulin. Essentially all A-II in all density subfractions was precipitated with either immunoglobulin. Particles from each density subfraction precipitated with anti-A-II immunoglobulin had an A-I/A-II molar ratio of approximately 2.0 (range 1.9-2.3). However, particles precipitated with anti-A-I immunoglobulin had A-I/A-II molar ratios identical to the A-I/A-II ratio of the subfraction (range 2.1-7.1). The subfractions with A-I/A-II molar ratios of about 2 had the least proportion of A-I in particles containing A-I but not A-II. Conversely, the subfractions with the highest A/I/A-II molar ratio had the greatest proportion of apoA-I in particles containing A-I but not A-II. These data indicate that HDL contains at least two types of particles: particles with both A-I and A-II in a 2:1 molar ratio, and particles containing A-I but no A-II. The variation in A-I/A-II ratio observed in different HDL density subfractions was due to the different proportions of these types of particles.

Cheung, M. C. and *Albers, J. J.*

Journal of Lipid Research 23:747-753, 1982.

Other support: National Institutes of Health.

From the Northwest Lipid Research Clinic and Department of Medicine, University of Washington School of Medicine, Seattle.

CULTURED ENDOTHEL
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